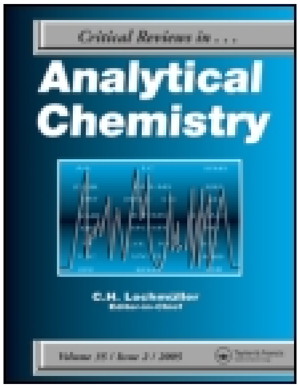

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/batc20

Flavonoids as Analytical Reagents

Krystyna Pyrzynska [a] & Anna Pękal [a]

[a] Department of Chemistry, Warsaw University, Warsaw, Poland

To cite this article: Krystyna Pyrzynska & Anna Pękal (2011): Flavonoids as Analytical Reagents, Critical Reviews in Analytical Chemistry, 41:4, 335-345

To link to this article: http://dx.doi.org/10.1080/10408347.2011.607077

Critical Reviews in Analytical Chemistry, 41:335–345, 2011
Copyright © Taylor and Francis Group, LLC
ISSN: 1040-8347 print / 1547-6510 online
DOI: 10.1080/10408347.2011.607077

Flavonoids as Analytical Reagents

Krystyna Pyrzynska and Anna Pękal
Department of Chemistry, Warsaw University, Warsaw, Poland

Compounds from the flavonoid family, while exhibiting a wide range of biological effects, are interesting reagents for analytical purposes as well. They form complexes with several *p*-, *d*-, and *f*-electron metals, which could be employed in the determination of these metal ions in different kinds of samples by various techniques. This article presents and discusses the application of flavonoid compounds as chromogenic agents in spectrophotometric and fluorometric detection, as complexing modifiers in solid phase extraction for preconcentration and separation of metal ions, and in adsorptive voltammetry for the determination of metals. Selected applications are included to illustrate the scope and limitations of the various approaches.

Keywords Flavonoids, chromogenic agents, complexing modifiers

INTRODUCTION

Flavonoids, benzo-γ-pyron derivatives with several hydroxyl groups attached to ring structures C_6-C_3-C_6, are one of the most important groups of compounds occurring in plants. They can be categorized as flavonols, flavanones, flavones, anthocyanidins, and isoflavones (Figure 1). Flavonoids exhibit a wide range of biological effects, including antibacterial, anti-inflammatory, anti-allergic, and antithrombotic actions (Boots et al., 2008; Aherne and O'Brien, 2002). Epidemiological studies point to their possible role in preventing cardiovascular diseases and cancer. Flavonoids behave as antioxidants in a variety of ways, including direct trapping of reactive oxygen species, inhibition of enzymes responsible for superoxide anion production, chelation of transition metals involved in processes forming radicals, and prevention of the peroxidation process by reducing alkoxyl and peroxyl radicals (Heim et al., 2002). Generally, by chelating metal ions, flavonoids prevent metal-catalyzed free radical generation and their subsequent reactions and accordingly protect very important biologically active molecules from oxidative stress (Fiorani et al., 2002).

Experimental data indicate that chelated compounds are more effective free radical scavengers than flavonoids alone (Kostyuk et al., 2001; Pękal et al., 2011). For example, complexes of rutin and green tea epicatechins with Fe(II), Fe(III), and Cu(II) are more effective radical scavengers than the corresponding free flavonoids due to the acquisition of additional superoxide dismutating centers (Kostyuk et al., 2001). Complexes of Cd(II) and Mn(II) with quercetin showed significant activity as a bactericide against *B. cereus* whereas sodium peniclinate exhibited no activity (Bravo and Anacona, 2001).

All types of flavonoids possess three domains able to react with metal ions: the 3′-4′-dihydroxy system located on the B ring and the 3-hydroxy or 5-hydroxy groups and 4-carbonyl group in the C ring (Figure 2). Cornard and Merlin (2002) have reported that in acidic conditions, the 3-hydroxy-4-ketone or the 5-hydroxy-4-ketone groups of quercetin are involved in complex formation with the stoichiometry of Me:L = 1:1. In alkaline media, the second implicated site is the 3′,4′-dihydroxy group located on the B ring, which allows formation of high stoichiometry complexes (1:2). For steric reasons the complexes usually include no more than two flavonoid molecules, however, four different Al-quercetin stoichiometries have been proposed, 1:1, 1:2, 1:3, and 2:3 (Cornard and Merlin, 2002; De Souza and De Giovani, 2005). It was demonstrated that the degree of complex formation was dependent on the Al-ligand ratio and the solvent system employed; in general, methanol favored formation of the 1:2 dimer, whereas dimeric complex was suppressed in acetonitrile and *i*-propanol. The formation of 3:1 complexes of morin (Woźnicka et al., 2007) and quercetin (Zhou et al., 2001) with rare-earth metal ions has been reported.

Flavonoids act as weak polybasics, so pH plays an import ant role in complex formation. The optimal pH for complex formation is around 6, although it strongly depends on the metal ion. At pH below 3.0, flavonoids remain undissociated, which is unfavorable for complex formation. At high pH values flavonoids are deprotonated and form more complex species. Furthermore, at higher pH values metal ions cause side reactions (hydrolysis) and hydroxocomplexes are formed (Grazul and Budzisz, 2009).

Spectroscopic studies indicated the reaction of quercetin with Cu(II) resulted in the formation of 1:1 metal-ligand

---

Address correspondence to Krystyna Pyrzynska, Dept. of Chemistry, Warsaw University, Pasteura 1, 02-093 Warsaw, Poland. E-mail: kryspyrz@chem.uw.edu.pl

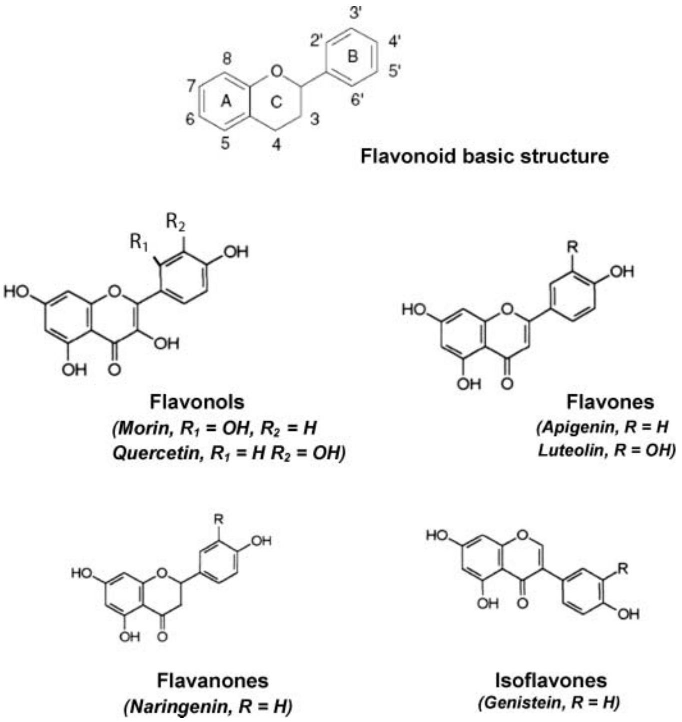

FIG. 1. Chemical structures of the common classes of flavonoids. Representative compounds are shown in brackets.

complex through the carbonyl oxygen and 3-OH group in the C ring (Pękal et al., 2011). Then quercetin is oxidized to the benzoquinone-type products and Cu(II) is rapidly reduced to Cu(I). The addition of ethylenediamine tetraacetic acid (EDTA) destroyed the complex but did not regenerate the whole original spectrum of quercetin. On the other hand, the presence of EDTA inhibits formation of that complex and quercetin oxidation. Fernandez et al. (2002) reported that quercetin does not appear to form complexes with Cu(I). The higher reducing capacity of quercetin for Cu(II) than for Fe(III) has been mentioned in a few studies (Fernandez et al., 2002; Mira et al., 2002; El Hajji et al., 2006). The redox properties of flavonoids largely depend on the number and position of hydroxy groups in the molecule (Brown et al., 1998).

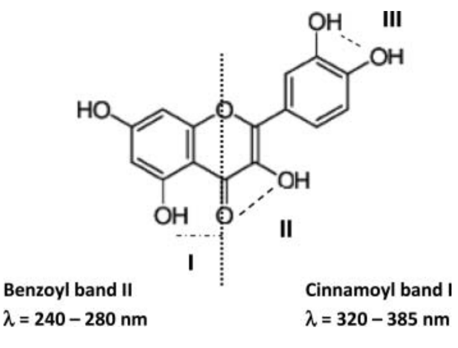

FIG. 2. Schemes of possible sites for complexation of metal ions with quercetin and the division of band I and II related to UV-VIS absorption bands.

The ability of flavonoids to form complexes with some *p*-, *d*-, and *f*-electron metals makes them interesting reagents for analytical purposes. The formed chelate complexes serve as a basis of the determination of metals by various techniques. This article discusses the application of flavonoid compounds as chromogenic agents in spectrophotometric and fluorometric detection, as complexing modifiers in solid phase extraction for preconcentration and separation of metal ions, and in adsorptive voltammetry for the determination of metals.

CHROMOGENIC AGENTS

Flavonoids exhibit two major absorption bands in the ultraviolet/visible region (Figure 2). The absorptions in the 320–385 nm range correspond to the B ring portion (cinnamoyl system, band I) and the absorptions in the 240–280 nm range correspond to the A ring portion (benzoyl system, band II). The spectra are related to the $\pi \rightarrow \pi^*$ transitions within the aromatic ring of the ligand molecules. In comparison with flavonoid absorption spectra, those of the metal complexes are shifted to the long-wavelength region. Such bathochromic shift can be explained by the extension of the conjugated system with the complexation (De Souza and De Giovani, 2005).

The complexes formed make the basis of the determination of metals by UV-VIS spectrophotometry and spectrofluorimetry. Morin and quercetin serve mainly as the chromogenic agents in analytical procedures for determination of Al(III) (Ahmed and Hossan, 1995; Lian et al., 2003a,b; Al-Kindy et al., 2003; Safvi et al., 2003; Saad et al., 2002), Cr(III) (El-Sayed et al., 2000; El-Sayed and Khalil, 1996), W(VI) (El-Sayed et al., 2000), Fe(III) (El-Sayed and Khalil, 1996), Ge(IV) (García-Campaña et al., 2001), Zr(IV) (Saad et al., 2002; Wang et al., 2000), Hf(IV) (Wang et al., 2000), and Mo(VI) (El-Sayed and Khalil, 1996). In contrast to these flavonoids, their sulfonate derivatives are highly soluble in water. The presence of sulfonate groups does not considerably change the acidic properties of –OH groups but increases the acidic properties of the molecules (Kopacz, 2003). This can be considered as an advantageous property due to the fact that it precludes the possibility of metal ion hydrolysis. Maximum absorption bands of the complexes formed mostly occur in the range of 400–470 nm with molar absorptivities (ε) at the level of 10^3–10^4 L/mol cm.

Aqueous micellar media are often used to solubilize complexes and to enhance the sensitivity of metal detection (Safvi et al., 2003; Saad et al., 2002; El-Sayed et al., 2000; El-Sayed and Khalil, 1996; García-Campaña et al., 2001). In some cases substantial increase in the value of molar absorptivities up to 10^5 L/mol cm level was observed (Saad et al., 2002; El-Sayed et al., 2000). The spectrophotometric and fluorimetric methods are precise and reliable, but flavonoids are not selective reagents. To minimize the interfering action of other metal ions present in natural samples, typical masking agents such as tartrates (Ahmed and Hossan, 1995; Saad et al., 2002), 1,10-phenantroline and hydroxylamine (Al-Kindy et al., 2003), as well as fluorides and thiourea (Saad et al., 2002) were used. In some cases a preliminary step before determination for the enrichment and separation of the analyte has been proposed (Hosseini et al., 2009; Escriche and Hernandez, 1985).

Application of the resonance Rayleigh scattering (RRS) determination of trace amounts of aluminum in natural waters and biological samples has been reported (Long et al., 2001). The method was based on the formation of a ternary Al(III)-morin-surfactant complex with the maximum scatter peaks at 476 nm for cetyltrimethylammonium bromide as a surfactant. It was found that this system showed two optimal pH values—one in the acidic medium and the other in the alkaline region—depending on the different affinities of Al(III) for organic ligands. Thus, the RRS method was used for the analysis of Al speciation in natural water samples. The labile monomeric Al fraction was determined at pH 5.8, while the total monomeric Al fraction was quantified at pH 9.1.

A reversed-phase high-performance liquid chromatography (HPLC) with fluorescence detection for the determination of labile monomeric Al(III) has been developed through pre-column complexation with morin (Lian et al., 2003a,b). The highly fluorescent aluminum-morin complex was separated on a Spherisorb ODS 2 column with an eluent consisting of methanol-acidified water (30/70, v/v) eluent. The most remarkable point of this procedure was that only the most toxic aluminum species, e.g., positively charged monomeric aquo- and hydroxy-Al species plus the complexes with weak phenolic ligands such as salicylic and caffeic acids, selectively respond among different aluminum complexes. This strategy has been applied to direct fractionation of this metal in natural waters and biological samples without sample pretreatment. Figure 3 shows the obtained chromatograms for different kinds of samples (Lian et al., 2003b).

Recent studies on the interaction of quercetin and morin with noble metals have shown that in hydrochloric acid solution the redox reaction occurs. The introduction of Au(III) (Balcerzak et al., 2006), Ru(IV) (Balcerzak et al., 2004), or Os(VIII) (Kosiorek-Rupińska et al., 2006) into a solution of these flavonoids results in the transformation of ligands into a new species exhibiting a stable absorption band at 291 nm. This band corresponds to the oxidized form of flavonoids identified as quinone. Similar reaction products were obtained when quercetin was replaced by its sulfonate derivative (quercetin-5′ sulfonic acid) (Balcerzak et al., 2004). No interaction of Pd(II) and Pt(IV) with flavonoid reagents has been observed in chloride solutions. The amount of the generated oxidized form of quercetin or morin corresponds to the concentration of Rh, Au, and Os in a solution, and it was used as the basis of a spectrophotometric method for the determination of this metal (Balcerzak et al., 2006; Balcerzak et al., 2004; Kosiorek-Rupińska et al., 2006). The calculation of the first-derivative spectrum allowed reaching a better detection limit (0.01 μg/mL) for determination of Os(VIII) than with direct spectrophotometry (0.1 μg/mL) (Kosiorek-Rupińska et al., 2006). Moreover, as chlorocomplexes of Os(IV) do not react with quercetin, it was possible to make speciation analysis of this metal. The signal of $OsCl_6^{2-}$ was isolated from the mixture by the calculation of the third-derivative spectrum. The reaction of Au(III) with quercetin and morin proceeds significantly slower than that when Ru(IV) was used as oxidant. The increase in temperature resulted in a shorter reaction time, e.g., 30 min at 70°C (Balcerzak et al., 2006; Balcerzak et al., 2004).

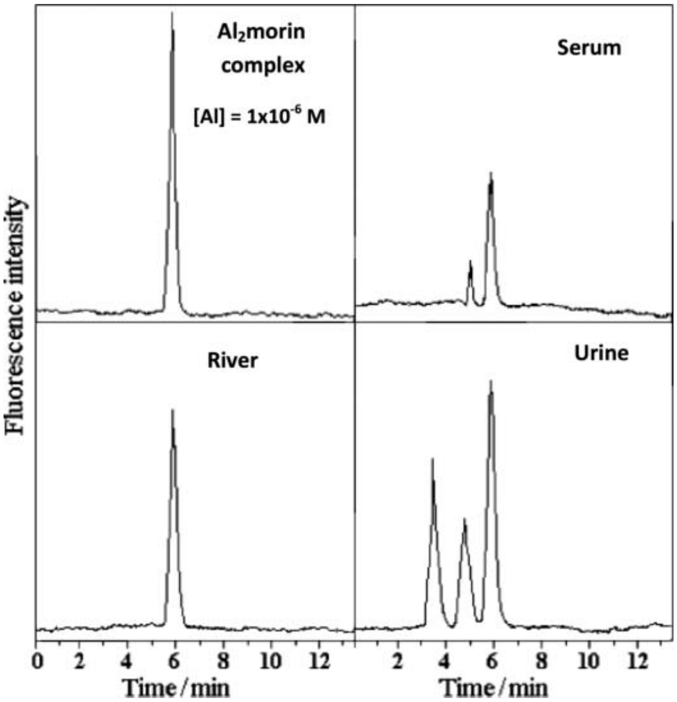

FIG. 3. Chromatograms of Al.-morin complex in different kinds of samples (Lian et al., 2003b).

A highly sensitive method has been proposed for direct speciation determination of trace amounts of chromium in water samples using quercetin (Hosseini and Belador, 2009). The mixing of quercetin (in *n*-amyl alcohol) is accomplished with luminescence quenching of the organic phase due to partial oxidation of flavonoid content. Cr(VI) is determined by measuring the diminished content of quercetin with the spectrofluorometric method ($\lambda_{ex} = 276$ nm, $\lambda_{em} = 331$ nm). After oxidation of Cr(III) to Cr(VI) and determination of total chromium, the content of Cr(III) could be obtained by subtracting. No considerable interference was observed due to the presence of coexisting cations and anions. Redox reaction between Fe(III) and flavonoids (morin, quercetin) was also applied for spectrophotometric determination of Fe(III) in Fe(II) samples (Balcerzak et al., 2008). The detection limits were 0.06 μg/mL and 0.38 μg/mL when using quercetin and morin, respectively. Any redox reaction between Fe(III) and myricetin (with 5 –OH groups) was not observed (Sungur and Uzar, 2008). Instead of this, Fe(III) formed complexes with this flavonoid in a 1:2 molar ratio at pH 4 ($\lambda_{max} = 420$ nm) and in a 1:1 molar ratio at pH 6 ($\lambda_{max} = 440$ nm) with the stability constant values of 2.5 $\times 10^9$ and 3.6 $\times 10^5$, respectively.

Selected examples of the application of morin and quercetin as chromogenic agents to the determination of metal ions are presented in Table 1.

COMPLEXING MODIFIERS IN SOLID PHASE EXTRACTION

Despite continuous progress in the detectability of instrumental methods for analysis, a direct determination of trace metal ions in environmental samples is still very often difficult because of insufficient sensitivity and selectivity of the methods used. Therefore, pretreatment steps in analytical procedures, such as preconcentration and/or selective separation of the analyte before its determination, are frequently necessary in order to reduce the effect of interference present in the matrix. Among the different techniques that can be used for this purpose, solid-phase extraction (SPE) using various sorbents has been developed very intensively in recent years (Pyrzynska and Trojanowicz, 1999; Camel, 2000; Pyrzynska, 2010). SPE offers a number of important advantages compared to classical liquid-liquid extraction such as higher enrichment factors, better recoveries, less consumption of organic solvents, quicker phase separation with the absence of emulsion, and more scope for coupling to different detection techniques.

Many materials, such as divinylbenzene polymers, modified silica gels, carbon-based sorbents, and polyurethane foams could be employed in SPE for separation/preconcentration purposes (Augusto et al., 2010; Lemos et al., 2007; Prasado Rao et al., 2004; Jal et al., 2005). Complexing resins are especially interesting due to their higher selectivity than single polymeric matrices. Complexing reagents can be introduced into the sorbent by two different means: (i) the chemical bonding of these reagents on existing sorbents (functionalized sorbents), and (ii) the physical binding of the chelate ligand on the sorbent surface (modified or loaded sorbent). Physical binding is the most simple to use in practice. However, chemical bonding allows elevated lifetime for the column due to covalent bonds between the ligand and the support. This property avoids the possible flushing and loss of the complexing regent from the column during the elution step.

Synthesis of chelating resins containing a covalently bound flavonoid molecule as a functional group has been mostly conducted using silicas matrices (Szczepaniak and Szymański, 1996; Azeredo et al., 2002; Abou-El-Sherbini and Hassanien, 2004; Zaporozhets et al., 2000, 2001; Hassanien and Abou-El-Sherbini, 2006). The functionalization step was usually performed via the Mannich-type reaction (Figure 4). Quercetin immobilized on silica gel has been successfully applied for enrichment of Mo(VI) in a slightly acidic solution (pH = 5.0) (Azeredo et al., 2002) as well as of Be(II) at pH 7 (Szczepaniak and Szymański, 1996). Synthesis of controlled-pore silica glass functionalized with quercetin was reported by Abou-El-Sherbini and Hassanien (2004). This sorbent was used for the separation and preconcentration of Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) at pH 7.5–8.5. The sorption capacity for these metal ions were in the range of 0.24–0.46 mmol/g, indicating a 1:2 quercetin-metal chelation for all metal ions except for Mn(II), for which a 1:1 ratio was suggested. Silica gels with immobilized quercetin (Zaporozhets et al., 2001) and morin (Zaporozhets et al., 2000) were used for sorption-spectrophotometric determination of Sn(IV) and Zr(IV), respectively.

A morin chelating resin was synthesized using aminated poly(vinyl chloride) as a starting material (Luo et al., 1992). It showed high affinity for Mo(VI) and W(VI) ions at pH 2. A similar affinity was also exhibited for Fe(III), but the interference from the presence of these ions can be eliminated by addition of ascorbate. The capacities of the resin were 4.17 mmol/g and 0.762 mmol/g for molybdenum and tungsten, respectively.

ADSORPTIVE VOLTAMMETRY FOR THE DETERMINATION OF METALS

Adsorptive stripping voltammetry (AdSV) is not only an extremely sensitive electrochemical technique for determination of trace metals in various matrices but also exhibits high selectivity. The formed metal chelates are adsorbed and accumulated on the working electrode by a non-electrolysis process. Then, depending on the oxidation-reduction properties of the accumulated analyte, the determination is carried out by scanning the potential in the appropriate negative or positive direction. The peak potential (E_p) is characteristic of the given metal and it can be used for qualitative identification, whereas the peak current (I_p) is proportional to the concentration of the corresponding analyte. Since stripping curves/peaks for various analytes occur at characteristic potentials, several metal species can often be determined simultaneously.

A mercury film electrode and a hanging mercury drop electrode have been traditionally used for AdSV because of the advantageous analytical properties of mercury in the negative potential range; however, in recent years the most popular schemes used to substitute mercury electrodes include carbon paste electrodes, polymer film electrodes, and carbon nanotube electrodes (Economou, 2010; Zima et al., 2009).

From the flavonoid family, morin is the most frequently used as a complexing reagent for trace amount determination of metal ions by AdSV (El-Maali et al., 1997; Zhou et al., 1998; Hajian and Shams, 2003, 2006; Liu et al., 2000; Shams et al., 2004) (Table 2). Except for the possibility to determine a single metal ion with high sensitivity, this technique was successfully used for simultaneous determination of bismuth and copper (Hajian and Shams, 2003) as well as copper, zinc, and lead (Shams et al., 2004) in some natural and synthetic samples with satisfactory results. Aranciba et al. (2009) applied morin-5′-sulfonic acid and sodium dodecyl sulfate (SDS) for determination of lead by AdSV. The sulfonic group of the ligand, which does not participate directly in the metal-ligand bond, contributes a

TABLE 1
Selected examples of application of morin and quercetin as a chromogenic agents for determination of metal ions

Metal ions	Conditions	λ_{max} nm	$\varepsilon \times 10^3$ L/mol• cm	Dynamic range μg/mL	Detection limit μg/L	Sample	Remarks	Ref.
Morin								
Al(III)	50% (v/v) EtOH	421	5.3	0.01–5	6.0	Alloys, environmental waters, biological samples	Absorbance remains stable for 48 h. Tartrates prevent interference from Pb(II), Fe(III), Zn(II), Ni(II), Ca(II), Hg(II).	Ahmed and Hossan, 1995
Al(III)	pH 4.5	ex 418 em 490		0.0001–1.6	0.16	Waters, tea infusions, bovine serum, human hair	Pre-column complexation for HPLC separation	Lian et al., 2003b
Al(III)	pH 4.5	ex 418 em 490		0.0001–1.6	0.16	Natural waters	Fractionation of Al(III) species	Lian et al., 2003b
Al(III)	Surfactant Tween-20	ex 425 em 495		50–1, 000	3.0	Tap water	1,10-phenantroline and hydroxylamine were added to decrease interference from Cu(II) and Fe(III).	Al-Kindy et al., 2003
Cr(III)	pH 3.4–4.2 EFA	435	113	0.03–0.46	16	Steel, wastewater, nonferrous alloys	Maximum absorbance was attained after 10 min at 90°C	El-Sayed et al., 2000
W(VI)	pH 1.9–2.5 PVP	415	21.3	0.7–8.1	630	Steel	Absorbance developed immediately and remained constant for more than 2 h.	El-Sayed et al., 2000
Fe(III)	CPB	412		0.9–1.5	40	Alloys	Full color development is obtained after 10 min.	El-Sayed et al., 1996
Mo(VI)		417		0.3–4.2	200			

(Continued on next page)

TABLE 1
Selected examples of application of morin and quercetin as a chromogenic agents for determination of metal ions *(Continued)*

Metal ions	Conditions	λ_{max} nm	$\varepsilon \times 10^3$ L/mol● cm	Dynamic range μg/mL	Detection limit μg/L	Sample	Remarks	Ref.
Au(III)	pH 2–3	291	20.2	0.2–12	200	Cosmetic cream samples	Heating at 70°C for 30 min. Separation of Cu(II) and Zn(II) are required.	Balcerzak et al., 2006
Fe(III)	0.3 M HCl	293	4.8	0.4–15	420	Pharmaceutical products	Heating at 70°C for 15 min. Fe(II) does not interfere.	Balcerzak et al., 2008
Quercetin								
Cr(III)	pH 4–4.6 CBP	441	102	0.14–2.1	920	Steel, wastewater, nonferrous alloys	Heating at 90°C for 10 min is required.	El-Sayed et al., 2000
Cr(VI)		292		0.04–3.2	0.4	Natural waters	After sorption of Cr(VI) by modified Amberlite XAD-7.	Saad et al., 2002
Zr(IV)	Oxalate + PVP	320	73.1	up to 1.46	60	Industrial wastewaters	Fluorides, tartrate, and thiourea were used as masking agents.	Saad et al., 2002
Al(III)	PVP	433	80.9	0.03–0.43	2			
Ge(IV)	Dodecyl ether	ex 432 em 552		7.4–150	7.4	Drugs, wholemeal oats	Fluorescence intensity reaches a maximum in a few seconds.	García-Campaña et al., 2001
Ru(IV)		291	5.0	0.11–30	60	Cosmetic cream	Quercetin-5′-sulfonic acid reacts in aqueous solution and much faster than quercetin.	Balcerzak et al., 2004
Au(IIII)			22	0.06–12				
Os(VIII), Os(IV)	0.05 M HCl	291	25.1	up to 11	10	K_2OsCl_6 reagent	Speciation analysis of osmium.	Kosiorek-Rupińska et al., 2006
Fe(III)	0.3 M HCl	291	7.5	0.1–15	70	Pharmaceutical products	Fe(II) does not interfere. Heating at 70°C for 15 min.	Balcerzak et al., 2008

EFA: etoxylated fatty alcohol; PVP: polyvinylpyrrolidone; CPB: cetylpyridinium bromide.

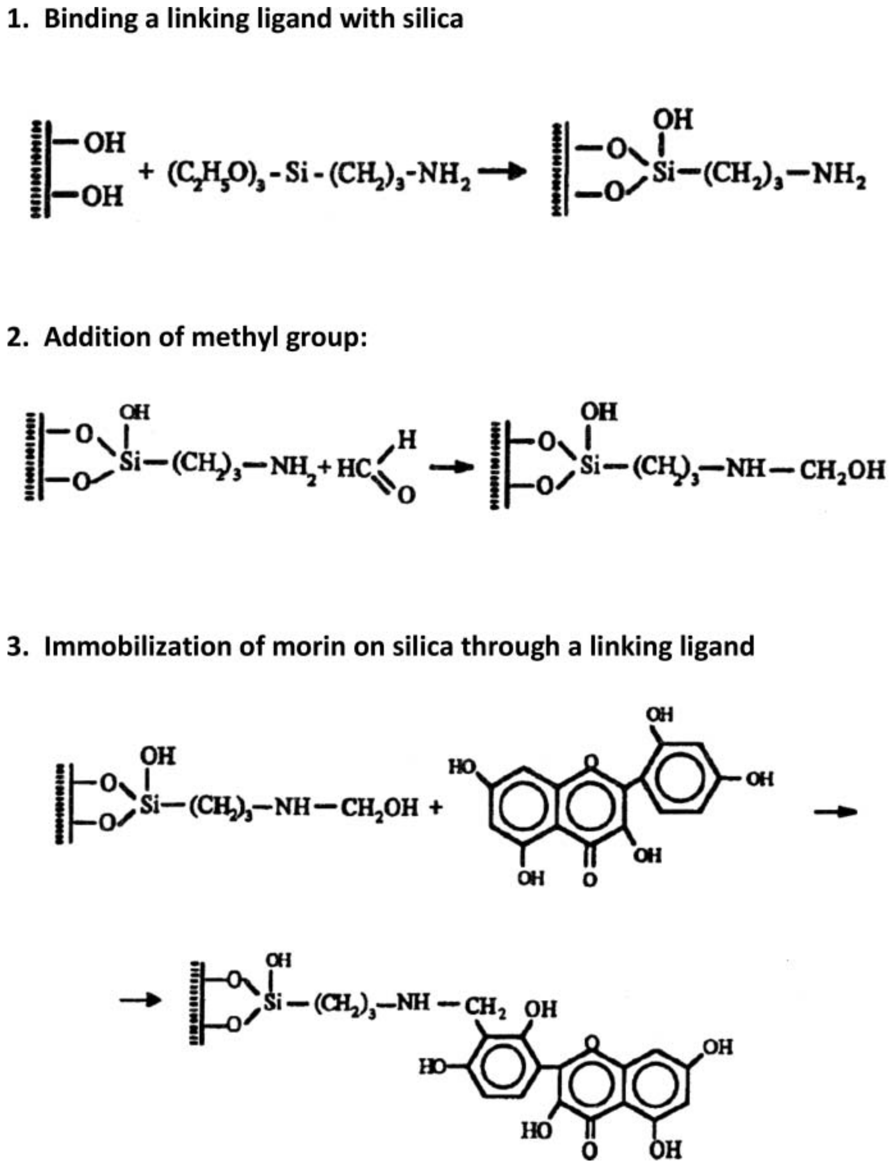

FIG. 4. Scheme for the immobilization of morin on silica gel (based on Szczepaniak and Szymański, 1996).

negative charge, which strengthens the bond with the surfactant and then with the electrode. The formed complex was accumulated at the surface of the hanging mercury drop electrode and after reduction at −0.48 V peak current was measured by square wave voltammetry. In the presence of small amounts of SDS, the peak current of the complex was increased. The possible mechanism of this enhancement effect involves the formation of negatively charged micelles of SDS with the lead complex and the positive charge of the mercury electrode to potentials of about −0.40 V. The detection limit of 0.04 μg/L was obtained and this method was applied to the determination of Pb(II) in tap and sea water samples after UV digestion (Hajian and Shams, 2006).

A series of new quercetin-modified carbon paste electrodes (Qu/CPE) were fabricated and investigated for simultaneous determination of copper, lead, and zinc based on their voltammetric response (Xia et al., 2010). Compared with Qu/CPE and quercetin ionic liquid–modified carbon paste electrodes (Qu-IL/CPE), the carbon paste electrode modified with hexagonal mesoporous silica (HMS) immobilized quercetin (HMSQu/CPE) exhibited higher sensitivity and selectivity toward the detection of studied metal ions due to high surface area, numerous active sites, and strong adsorption ability of HMS (Figure 5). Detection limits of 0.32 μg/L, 0.17 μg/L, and 0.11 μg/L for Cu(II), Pb(II), and Cd(II) were obtained, respectively. This novel electrochemical method was applied to the analysis of soil samples.

TABLE 2
Selected examples of application of adsorptive stripping voltammetry and morin for determination of metal ions

Metal ions	Detection/stripping mode	Working electrode	Conditions	Accumulation potential, V	Sample	LOD μg/L	Ref.
Al(III),	CSV	SMDE	Acetate/HEOES buffer	-1.1^{a}	Portland cement	0.11	El-Maali et al.,
Fe(III)			pH 8.5	-0.2^{a}		0.34	1997
Sb(III)	CSV	SMDE	Chloroacetic acid pH 2.3	-0.23^{b}	Steel, brass	0.85	Zhou et al., 1998
Bi(III)	CSV	HMDE	0.1–0.15 M	-0.1^{a}	Tap water, copper alloy	4.5	Hajian and
Cu(II)			HNO_3	-0.3^{a}		0.6	Shams, 2003
Zr(IV)	ASV	MCPE	2.2 M HCl	0.0^{b}	Ore samples	0.91	Liu et al., 2000
Cu(II)	CSV	HMDE	Acetate buffer	-0.5^{a}	Tap water, alloys	0.06	Shams et al., 2004
Zn(II)			pH 4			0.08	
Pb(II)						0.06	
Mo(VI)	SWV	HMDE	0.15 M HNO_3	-0.35^{a}	Tomato, cucumber, tea	0.45	Hajian and Shams, 2006

[a] vs. Ag/AgCl (saturated KCl).

[b] vs. SCE.

CSV: cathodic stripping voltammetry; ASV: anodic stripping voltammetry; SWV: square-wave voltammetry; SMDE: static mercury drop electrode; HMDE: hanging mercury drop electrode; MCPE: modified paste electrode; HEPES: 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethane.

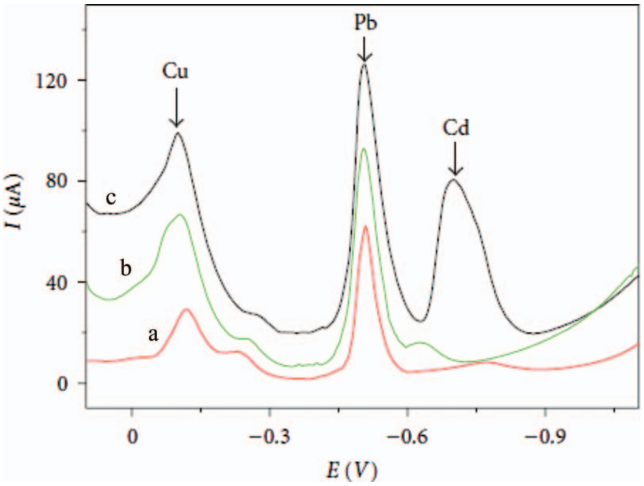

FIG. 5. Differential pulse voltammograms of 1.0 μM (each) multicomponent Cu(II)/Pb(II)/Cd(II) solution at: (a) quercetin-modified carbon paste electrode (Q/CPE); (b) quercetin ionic liquid–modified carbon paste electrode Qu-IL/CPE; and (c) carbon paste electrode modified with hexagonal mesoporous silica immobilized quercetin (HMS-Qu/CPE). Conditions: 0.1 M HCOONa-HCl (pH 4.7); preconcentration potential 0.6 V; preconcentration time 120 s (Xia et al., 2010). (Figure available in color online.)

CONCLUSIONS

Compounds from the flavonoid family, in addition to exhibiting a wide range of biological effects, are interesting reagents for analytical purposes. They form complexes with several *p*-, *d*-, and *f*-electron metals that could be employed in the determination of these metal ions in different kinds of samples by various techniques. The application of flavonoids as chromogenic reagents in spectrophotometric and fluorimetric detection allows developing very sensitive analytical procedures for metal quantification with molar absorptivities in the range of 10^3–10^5 L/mol cm. The highly fluorescent aluminum-morin complex was applied to chromatographic analysis and direct fractionation of this metal in natural waters and biological samples without sample pretreatment. The redox reactions between flavonoids and some metal ions at higher valence state were utilized as a basis of the spectrophotometric method for the determination of these metals as well as their speciation analysis, as was shown in the case of iron, osmium, and chromium. Flavonoids could also be used as complexing modifiers in solid phase extraction for preconcentration and separation purposes to increase the sensitivity of analytical measurements and in adsorptive voltammetry for the determination of metals.

Among flavonoid compounds, morin and quercetin as well as their sulfonate derivatives are the most frequently used in the proposed analytical procedures, probably due to the high cost of other reagents. However, the complexes of metal ions with other flavonoids such as rutin (De Souza and De Giovani, 2005; Bai et al., 2004), luteolin (Jungbluth et al., 2000), myricetin (Sungur and Uzar, 2008; Jungbluth et al., 2000), genistein (Dixon and Ferreira, 2002), and naringenin (Wang et al., 2006) have also been synthesized and characterized. In the future they will probably be employed more in analytical procedures.

REFERENCES

Abou-El-Sherbini, K. S.; Hassanien, M. M. Synthesis of Controlled-Pore Silica Glass Functionalized with Quercetin and Its Application for the Separation and Preconcentration of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II). *Sep. Sci. Technol.* **2004**, *39*, 1177–1201.

Aherne, S. A., O'Brien, N. M. Dietary Flavonols: Chemistry, Food Content, and Metabolism. *Nutrition* **2002**, *18*, 75–81.

Ahmed, M. J.; Hossan, J. Spectrophotometric Determination of Aluminium by Morin. *Talanta* **1995**, *42*, 1135–1142.

Al-Kindy, S. M. Z.; Suliman, F. O.; Salama, S. B. A Sequential Injection Method for the Determination of Aluminum in Drinking Water Using Fluorescence Enhancement of the Aluminum-Morin Complex in Micellar Media. *Microchem. J.* **2003**, *74*, 173–179.

Aranciba, V.; Nagles, E.; Cornejo, S. Determination of Lead in the Presence of Morin-5′-Sulfonic Acid and Sodium Dodecyl Sulphate by Adsorptive Stripping Voltammetry. *Talanta* **2009**, *80*, 144–188.

Augusto, F.; Carasek, E.; Costa Silva, R. G.; Rivellino, S. R.; Batista, A. D.; Martendal, E. New Sorbents for Extraction and Microextraction Techniques. *J. Chromatogr. A* **2010**, *1217*, 2533–2542.

Azeredo, L. C.; Azeredo, M. A. A.; Castro, R. N.; Saldanha, M. F. C.; and Perez, D. V. Separation and Determination of Molybdenum by Inductively Coupled Plasma Optical Emission Spectrometry Using Quercetin Immobilization on Silica Gel. *Spectrochim. Acta Part B* **2002**, *57*, 2181–2185.

Bai, Y.; Song, F.; Chen, M.; Xing, J.; Liu, Z.; Liu, S. Characterization of the Rutin-Metal Complex by Electrospray Ionization Tandem Mass Spectrometry. *Anal. Sci.* **2004**, *20*, 1147–1151.

Balcerzak, M.; Kopacz, M.; Kosidorek, A.; Swiecicka, E.; Kus, S. Spectrophotometric Studies of the Interaction of Noble Metals with Quercetin and Quercetin-5′-Sulfonic Acid. *Anal. Sci.* **2004**, *20*, 1333–1337.

Balcerzak, M.; Kosiorek, A.; Swiecicka, E. Morin as a Spectrophotometric Reagent for Gold. *J. Anal. Chem.* **2006**, *61*, 119–123.

Balcerzak, M.; Trybulska, A.; Święcicka-Füchsel, E. Selective Determination of Fe(III) and in Fe(II) Samples by UV-Spectrophotometry with the Aid of Quercetin and Morin. *Acta Pharm.* **2008**, *58*, 327–334.

Boots, A.; Haenen, G. R. M.; Bast, A. Health Effects of Quercetin: From Antioxidant to Nutraceutical. *Eur. J. Pharmacol.* **2008**, *585*, 325–337.

Bravo, A.; Anacona, J. R. Metal Complexes of the Flavonoid Quercetin: Antibacterial Properties. *Transit. Met. Chem.* **2001**, *26*, –23.

Brown, J. E.; Khodr, H.; Hider, R. C.; Rice-Evans, C. A. Structural Dependence of Flavonoid Interactions with Cu^{2+} Ions: Implications for Their Antioxidant Properties. *Biochem. J.* **1998**, *330*, 1173–1178.

Camel, V. Solid Phase Extraction of Trace Elements. *Spectrochim. Acta Part B* **2000**, *58*, 1177–1233.

Cornard, J. P.; Merlin, J. C. Spectroscopic and Structural Study of Complexes with Quercetin with Al(III). *J. Inorg. Biochem.* **2002**, *92*, 19–27.

De Souza, R. F. V.; De Giovani, W. F. Synthesis, Spectral and Electrochemical Properties of Al(III) and Zn(II) Complexes with Flavonoids. *Spectrochim. Acta Part A* **2005**, *61*, 1985–1990.

Dixon, R. A.; Ferreira, D. Genistein. *Phytochemistry* **2002**, *60*, 205–211.

Economou, A. Recent Developments in On-Line Electrochemical Stripping Analysis—An Overview of the Last 12 Years. *Anal. Chim. Acta* **2010**, *683*, 38–51.

El Hajji, H.; Nkhili, E.; Tomao, V.; Dangles, O. Interactions of Quercetin with Iron and Copper Ions: Complexation and Autoxidation. *Free Radical Res/* **2006**, *40*, 303–320.

El-Maali, N. A.; Temerk, Y. M.; Al-Aziz, M. S. Application of Stripping Voltammetry at a Static Mercury Drop Electrode for the Determination of Aluminium and Iron in Portland Cement. *Anal. Chim. Acta* **1997**, *353*, 313–318.

El-Sayed, A. Y.; Khalil, M. M. H. Simultaneous First-Derivative Spectrophotometric Determination of Iron(III) and Molybdenium(VI) in Cobalt-Chromium and Nickel-Chromium Alloys. *Talanta* **1996**, *43*, 583–588.

El-Sayed, A. A. Y.; Saad, E. A.; Ibrahime, B. M. M.; Zaki, M. T. M. Flavonol Derivatives for Determination of Cr(III) and W(VI). *Microchim. Acta* **2000**, *135*, 19–27.

Escriche, J. M.; Hernandez, F. H. Fluorimetric Determination of Aluminium with Morin after Extraction with Isobutyl Methyl Ketone. Part II. Extraction—Fluorimetric Determination of Aluminium in Natural and Waste Waters. *Analyst* **1985**, *110*, 287–290.

Fernandez, M. T.; Mira, M. L.; Florencio, M. H.; Jennings, K. R. Iron and Copper Chelation by Flavonoids: An Electrospray Mass Spectroscopy Study. *J. Inorg. Biochem.* **2002**, *92*, 105–111.

Fiorani, M. T.; de Sancitis, R.; de Bellis, R.; Dacha, M. Intracellular Flavonoids as Electron Donors for Extracellular Ferricyanide Reduction in Human Erythrocytes. *Free Radic. Biol. Med.* **2002**, *32*, 64–72.

García-Campaña, A. M.; Barrero, F. A.; Lupiáñez González, A.; Román Ceba, M. Non-ionic Micellar Solubilization—Spectrofluorimetric Determination of Trace of Germanium(IV) with Quercetin in Real Samples. *Anal. Chim. Acta* **2001**, *477*, 219–228.

Grazul, M.; Budzisz, E. Biological Activity of Metal Ion Complexes of Chromones, Coumarins and Flavones. *Coord. Chem. Rev.* **2009**, *253*, 2588–2598.

Hajian, R.; Shams, E. Application of Adsorptive Stripping Voltammetry to the Determination of Bismuth and Copper in the Presence of Morin. *Anal. Chim. Acta* **2003**, *491*, 63–69.

Hajian, R.; Shams, E. Trace Determination of Molybdenum by Adsorptive Cathodic Stripping Voltammetry. *J. Iran. Chem. Soc.* **2006**, *3*, 32–37.

Hassanien, M. M.; Abou-El-Sherbini, K. S. Synthesis and Characterisation of Morin-Functionalised Silica Gel for the Enrichment of Some Precious Metal Ions. *Talanta* **2006**, *68*, 1550–1559.

Heim, K. E.; Tagliaferro, A. T.; Bobilya, D. J. Flavonoid Antioxidants: Chemistry, Metabolism and Structure-Activity Relationships. *J. Nutr. Biochem.* **2002**, *13*, 572–584.

Hosseini, M. S.; Belador, F. Cr(III)/Cr(VI) Speciation Determination of Chromium in Water Samples by Luminescence Quenching of Quercetin. *J. Hazard. Mater.* **2009**, *165*, 1062–1067.

Hosseini, M. S.; Bandegharaei, A. H.; Raissi, H.; Belador, F. Sorption of Cr(VI) by Amberlite XAD-7 Resin Impregnated with Brilliant Green and Its Determination by Quercetin as a Selective Spectrophotometric Reagent. *J. Hazard. Mater.* **2009**, *169*, 52–57.

Jal, P. K.; Patel, S.; Mishra, B. K. Chemical Modification of Silica Surface by Immobilization of Functional Groups for Extractive Concentration of Metal Ions. *Talanta* **2005**, *63*, 1005–1028.

Jungbluth, G.; Rühling, I.; Terns, W. Oxidation of Flavonols with Cu(II), Fe(II) and Fe(III) in Aqueous Media. *J. Chem. Soc. Perkin Trans.* **2000**, *2*, 1946–1952.

Kopacz, M. Quercetin and Morinsulfonates as Analytica Reagents. *J. Anal. Chem.* **2003**, *58*, 225–229.

Kosiorek-Rupińska, A.; Święcicka-Füschel, E.; Balcerzak, M. Speciation Analysis of Osmium(VIII) and Osmium(IV) by UV-VIS Spectrometry Using Quercetin as the Reagent. *Anal. Lett.* **2006**, *39*, 589–602.

Kostyuk, V. A.; Potapovich, A. I.; Vladykovskaya, E. N.; Korkina, L. G.; Afanasèv, I. B. A. Influence of Metal Ions on Flavonoid Protection against Asbestos-Induced Cell Injury. *Arch. Biochem. Biophys.* **2001**, *385*, 129–137.

Lemos, V. A.; Santos, M. S.; Santos, E. S.; Santos, M. J. S.; dos Santos, W. N. L.; Souza, A. S.; de Jesus, D. S.; das Virgens, C. F.; Carvalho, M. S.; Oleszczuk, N.; Vale, M. G. R.; Welz, B.; Ferreira, S. L. C. Application of Polyurethane Foam as a Sorbent for Trace Metal Pre-Concentration—A Review. *Spectrochim. Acta Part B* **2007**, *62*, 4–12.

Lian, H. Z.; Kang, Y. F.; Yasin, A.; Bi, S. P.; Shao, D. L.; Chen, Y. J.; Dai, L. M.; Tian, L. C. Determination of Aluminum in Environmental and Biological Samples by Reversed-Phase High-Performance Liquid Chromatography via Pre-Column Complexation with Morin. *J. Chromatogr. A* **2003a**, *993*, 179–185.

Lian, H. Z.; Kang, Y. F.; Bi, S. P.; Yasin, A.; Shao, D. L.; Chen, Y. J.; Dai, L. M.; Tian, L. C. Morin Applied in Speciation of Aluminum in Natural Waters and Biological Samples by Reversed-Phase High-Performance Liquid Chromatography with Fluorescence Detection. *Anal. Bioanal. Chem.* **2003b**, *376*, 542–548.

Liu, S.; Li, J.; Mao, X. Stripping Voltammetric Determination of Zirconium with Complexing Preconcentration of Zirconium(IV) at a Morin Modified Carbon Paste Electrode. **2000**, *15*, 1751–1755.

Long, X.; Bi, S.; Ni, H.; Tao, X.; Gan, N. Resonance Rayleigh Scattering Determination of Trace Amounts of Al in Natural Waters and Biological Samples Based on the Formation of an Al(III)-Morin-Surfactant Complex. *Anal. Chim. Acta* **2001**, *501*, 89–97.

Luo, X. Y.; Su, Z. X.; Gao, W. Y.; Zhan, G. Y.; Chang, X. J. Synthesis of a Morin Chelating Resin and Enrichment of Trace Amounts of Molybdenum and Tungsten Prior to Their Determination by Inductively Coupled Plasma Optical Emission Spectrometry. *Analyst* **1992**, *117*, 145–149.

Mira, L.; Fernandez, M. T.; Santos, M.; Rocha, R.; Florencio, M. H.; Jennings, K. R. Interactions of Flavonoids with Iron and Copper Ions: A Mechanism for Their Antioxidant Activity. *Free Radical Res.* **2002**, *36*, 1199–1208.

Pękal, A.; Biesaga, M.; Pyrzynska, K. Interaction of Quercetin with Copper Ions—Complexation, Oxidation and Reactivity towards Radicals. *Biometals* **2011**, *24*, 41–49.

Prasado Rao, T.; Praveen, R. S.; Daniel, S. Styrene-Divinylbenzene Copolymers; Synthesis, Characterization, and Their Role in Inorganic Trace Analysis. *Crit. Rev. Anal. Chem.* **2004**, *34*, 177–193.

Pyrzynska, K. Carbon Nanostructures for Separation, Preconcentration and Speciation of Metal Ions. *Trends Anal. Chem.* **2010**, *29*, 718–727.

Pyrzynska, K.; Trojanowicz, M. Functionalized Cellulose Sorbents for Preconcentration of Trace Metals in Environmental Analysis. *Crit. Rev. Anal. Chem.* **1999**, *29*, 313–321.

Saad, E. A.; Khalil, I. H.; Zaki, M. T. M.; El-Ella, A. A. Determination of Zirconium(IV) and Aluminum(III) in Waste Waters. *Microchim. Acta* **2002**, *140*, 87–91.

Safavi, A.; Mirzaee, M.; Abdollahi, H. Simultaneous Spectrophotometric Determination of Iron, Titanium, and Aluminum by Partial Least-Squares Calibration Method in Micellar Medium. *Anal. Lett.* **2003**, *36*, 699–712.

Shams, E.; Babaei, A.; Soltaninezhad, M. Simultaneous Determination of Copper, Zinc and Lead by Adsorptive Stripping Voltammetry in the Presence of Morin. *Anal. Chim. Acta* **2004**, *501*, 119–124.

Sungur, S.; Uzar, A. Investigation of Complexes Tannic Acid and Myricetin with Fe(III). *Spectrochim. Acta Part A* **2008**, *69*, 225–229.

Szczepaniak, W.; Szymański, A. Sorption and Preconcentration of Trace Amounts of Beryllium from Natural Waters on Silica Gel with Immobilized Morin Prior to Its Determination by ETA-AAS Method. *Chem. Anal. (Warsaw)* **1996**, *41*, 193–199.

Wang, Z. P.; Shi, L. L.; Chen, G. S.; Cheng, K. L. Multivariate Spectrofluorimetry of Ultra Trace Zirconium(IV) and Hafnium(IV) Assisted by Several Chemometric Methods. *Talanta* **2000**, *51*, 315–326.

Wang, H. L.; Yang, Z. Y.; Wang, B. D. Synthesis, Characterization and the Antioxidative Activity of Copper(II), Zinc(II) and Nickel(II) Complexes with Naringenin. *Transit. Met. Chem.* **2006**, *31*, 470–474.

Woźnicka, E.; Kopacz, M.; Umbreit, M.; Kłos, J. New Complexes of La(III), Ce(III), Pr(III), Nd(III), Sm(III), Eu(III) and Gd(III) Ions with Morin. *J. Inorg. Biochem.* **2007**, *101*, 774–782.

Xia, F.; Zhang, X.; Zhou, C.; Sun, D.; Dong, Y.; Liu, Z. Simultaneous Determination of Copper, Lead and Cadmium at Hexagonal Mesoporous Silica Immobilized Quercetin Modified Carbon Paste Electrode. *J. Autom. Methods Manag. Chem.* **2010**, article ID 824197.

Zaporozhets, O. A.; Ivan'ko, L. S.; Marchenko, I. V.; Sukhan, V. V. Determination of Zirconium Using Morin Immobilized on Silica Gel. *J. Anal. Chem.* **2000**, *55*, 540–544.

Zaporozhets, O. A.; Ivanko, L. S.; Marchenko, I. V.; Orlichenko, E. V.; Sukhan, V. V. Quercetin Immobilized on Silica Gel as A Solid Phase Reagent for Tin(IV) Determination by Using the Sorptive-Spectroscopic Method. *Talanta* **2001**, *55*, 313–319.

Zhou, Z. L.; Lu, Y.; Li, X. L.; Luo, C. N.; Zhang, Z. W.; You, J. M. Adsorptive Stripping Voltammetric Determination of Antimony. *Talanta* **1998**, *46*, 1531–1536.

Zhou, J.; Wang, L. F.; Wang, J. Y.; Tang, N. Synthesis, Characterization, Antioxidative and Antitumor Activities of Solid Quercetin Rare Earth(III) Complexes. *J. Inorg. Biochem.* **2001**, *83*, 41–48.

Zima, J.; Svancara, I.; Barek, J.; Vytras, K. Recent Advances in Electroanalysis of Organic Compounds at Carbon Paste Electrodes. *Crit. Rev. Anal. Chem.* **2009**, *39*, 204–227.